

# Overview of aloe vera



Aloe Vera is known as a TRUE ALOE. It is mostly used in herbal medicines so it is also known as FIRST AID PLANT or MEDICINAL PLANT. The ALOE word was derived from the aerobic word-' alloeh' which means " BITTER SUSSTANCES". Aloe is an emollient resin with the softening properties. It belongs to family Liliaceae [1].

### 1. 1. 1 HISTORY:-

Aloe vera was considered to one of best medicinal plants used in the treatment of various health conditions. It was first understood by Egyptians to having multiple nature of it properties [19]. But Aloe Vera was first discovered in 1862 by German Egyptologist -George Fbers. But its first English translation was found in 1655 by John Goodyear in Dioscorider' de materia medica, and he wrote aloe is very better in taste.

Aloe Vera has been used by many different cultures: Indians, Chinese, Greeks, Romans, have all used aloe Vera as a medicinal or healing plant. Botanists have find more than 300 species of aloe Vera like rumex, barbadensis etc. Out of this species, only five have strongest medical benefits likes Aloe barbadensis miller, Aloe perryi baker, Aloe ferox, Aloe saponaria , Aloe arborescens. Aloe barbadensis miller is mostly widely used and most potent as well. Throughout the history of aloe vera, the plant is in various form having tropical properties-refered as ' plant of immortality'. It is belonging to lily family because flower produce annually in the spring which resembles of ester lily. [1-2].

### 1. 1. 2 COMMON NAME:-

Chinese aloe, True aloe, Indian aloe, First aid plant, Medicinal plant, Miracle plant , Barbados aloe, Burn aloe and many more [1].

### 1. 1. 3 SCIENTIFIC NAME:-

Aloe barbadensis, Aloe capensis [1].

### 1. 1. 4 ALTERNATIVE NAME:-

Aloe Vera has a different name in worldwide like, In India-Ghrtakumari But in Gujarat and Rajasthan it's known as Gwarpatha, In Pakistan-quargandal which is used in unani medicine, In Indonesia-lidah buaya, In Thailand-crocodile tail plant, In south Africa-sabila [2].

### 1. 1. 5 DESCRIPTION:-

Aloe Vera grows in to the dreary climates or land. So it is mostly found in the India, Africa, Caribbean, and other dry climates.

Aloe Vera is a short stem or stems less plant. It mainly contains leaves and flowers. Aloe leaves are in green in colour and thick with the same variety. The size of leaves is 50-60 cm long and 4-5 cm thick. The margin of the leaves is serrated. But plants bear flowers once in a year in summer season. Flowers contain tubular collar which is yellow in colour and contain ' aloe tic' juice can be collected by cutting the leaves close to stem. The structure of aloe leaf shows an outer cortex which is hard duo to the present of calcium and magnesium. Tubes of xylem and phloem were found below the cortex and its supplied water and minerals to leaves [1].

### 1. 2 CHEMICAL CONSTITUTE:-

<https://assignbuster.com/overview-of-aloe-vera/>

Aloe Vera has weird medicinal properties. Botanists have found more than 200 important nutritional constituents in aloe Vera leaf which having to perform a function. They are MINERALS like Calcium, Sodium, Copper, Zinc, Iron, and Manganese (Essentials for bones, Regulates acidic or alkaline level of body fluid), VITAMINS like Vitamin A, Vitamin C, Vitamin E, Vitamin B12, and Folic Acid(To develop new blood cells), ENZYMES like lipase, Peroxidase (Helps indigestion), SUGAR like Monosaccharide's and polysaccharides include Sucrose, lactose(Maintain cholesterol level, Improve the strengthening of bones), LIGNINS which are the Cellular substances which hasn't medicinal benefits But it has an accessing property. AMINO ACID which are required by human body provided by aloe Vera like Leucine, Isoleucine, Valine, Theonine, Lysine, Methionine, Phenylamine, STEROL like Camp sterol, sitosterol(Pain killing properties), ANTHRAQUINONES like Aloin, Anthranol, Aloetic acid, Barbaloin, Isobarbaloin and Aloe emodin

Aloe emodine [4] Aloin [3]

And OTHERS like Salicylic acid, Tannins, Monosulfonic acid, Acemannam, Water [1-2].

Aloes Vera mainly contain 96-99% of water and constitutes are in rare part. So it's work in based on " SYNERGISTIC ACTIONS " which means ' working together of two or more substance and give greater action as compare to single substance. This phenomenon was explained by Dr. Atherton [1-2].

1. 3 CULTIVATION OF ALOE VERA [1-5]:-

Aloe Vera grown commercially for its high demand in cosmetic industries and treatment of healing in India. It has medicinal properties due to its bitter taste. Aloe Vera grown in a rainless climates or land. It's can't stay in cold temperature. So it is grown in all part of South Africa and India like Rajasthan, Gujarat, Maharashtra and many more.

Soil requirement for all species are coarse sandy loam soil with some fertility. In addition, it is noticed that its growth was easier and faster in black cotton soil in central India with the nearly by acidic ph.

For the perfect growth of Aloe Vera, lane should not distribute below the level of 20-30cm. Aloe Vera need a flat land and should be exact level with 2-3 farming. Plant should plant with some distance because of irrigation. Distance between two fields was 10m X 3m.

The proper time of suckers should be planted in month of July-August in monsoon season because suckers get proper water to grow up and survival. Suckers should have 3-4 month old having 3-4 leaves and 20-22 c long. About 30, 000 suckers needed for one hectare planting. After planting suckers, soil around the root should press and waste must be made proper to avoid the sluggishness.

Irrigation is necessary a critical stage to proper growth of plant. First irrigation is required after planting suckers followed by 4-6 per year which is reducing by every time. Weeding should be carried out after planting the plant and two or three weeding carried out throughout year.

All the species of Aloe Vera are insusceptible to most of insects and pests from any part of county. But sometime bugs, leaves dots have been reported in some part of country because of light irrigation.

Generally yield obtained from 2 to 5 year after plating. Average yield after 2 year is around 15-20 t/ha fresh leaves. Well managed irrigated crop give around 30-35 t/ha fresh leaves. Fully well developed leaves give an ' ALOE JUICE'.

After harvesting, marketing of Aloe Vera leaves is not fully developed in our country.

#### 1. 4 USES:-

Aloe Vera is well known for its medicinal properties since centuries. The plant is able to cure a wide range of disease. The part of plant like leaves, leaf gel is used clinically. Some respective uses of Aloe Vera are (1) EXTERNAL USES- Use in treatment of burns, allergic reaction, wounds, acne, Rheumatoid arthritis, Rheumatic fever, Acid indigestion cuts, Inflammatory condition of digestive system, Sun burn ulcers, Kill mould, bacteria, viruses, fungus, Wrinkles etc (2)COMMERCIAL USE: - Aloe Vera is widely use in cosmetics and many more like in ointments, pills, jelly, lotion, bevareges. as a foodstuff etc [1-6].

(3)MEDICINAL USE-They are Treatment of diabetes because it reduce blood sugar, Treatment of AIDS, Protect from lung cancer, Aloe juice is used for consumption issues, Prevent fungal infection, Prevent vaginal infection, Prevent scarring, Eczema, Constipation, Intestinal infection, Relieves from

candiala, Treatment of hyperglycaemia, Maintain level of cholesterol, Injection of Aloe Vera extracts to treat cancer, Skin disorder. And some of general use like Antibacterial properties, Antifungal properties, and Prevent radiation induced injuries, Inhibit growth of streptococcus species in vitro [1-6].

#### 1. 4. 1 ALOE PREPARATIONS: -

some of the aloe preparation which are widely use like aloe vera shampoo, aloe vera lotion, aloe vera gel, aloe vera juice, aloe vera eating, aloe vera butter, aloe vera dried herbs powders, aloe vera shower gel, and aloe vera vegetarian capsules [1].

#### 1. 4. 2 SIDE EFFECT AND SAFETY PRECAUTION: -

Aloe Vera has a medicinal or healing properties, it does come with some disadvantage. Aloe Vera gel, aloe Vera cream does not having major side effect but other preparation like juice, shampoo butter, having certain impediment like, Diarrhea, Blood electrolyte imbalance, Constipation, Muscle weakness, Abnormal heart rhythms [7]. Aloe Vera injection is to be avoided because of having fatalities. It can cause death with cancer patients Aloe Vera should be avoided while pregnancy, children, breast feeding [7].

#### ALPHA-GLUCOSIDES INHIBITORS: -

#### 1. 5 INTRODUCTION TO ALPHA-GLUCOSIDE ENZYME: -

It is a class of a medication for type-2 diabetes which decrease blood sugar level by decreasing carbohydrates from the intestinal. Discovery of alpha-

glucosides inhibitors has been very useful to develop therapeutic for the treatment for the carbohydrate- mediated disease like diabetes. Two classes of drugs like glycosidase inhibitors and lipase inhibitors which lower blood glucose by changing the absorption level of fat and carbohydrates [8-9].

Alpha - glucosides inhibitors like acarbose, miglitol, voglibose have been studies in Europe country but some of these are also available in united state [8]. Alpha -glucosidase inhibitors reduce the impact of carbohydrates blood sugar by inhibit the upper level of gastrointestinal [8].

#### 1. 5. 1 MECHANISM OF ACTION: -

Alpha glucosides inhibitors are competitive inhibitors of 1alpha glucosidase which are located in the brush border of small intestinal (epithelial cells). These inhibitors bind to the oligosaccharide binding site of the enzyme and delay digestion of polysaccharide resulting slow down food digestion in gut [10].

While the gastrointestinal tract dose not play a important role in the cure of diabetes, but changing its physiological activity can be used to control disease. In these case alpha-glucosides inhibitors are used. Alpha glucosides delay digestion of carbohydrates by hydrolyses of oligosaccharides into monosaccharide. Alpha glucosidase inhibitors can be used to reduce glycemic excussions and hypoglycaemia having type-1 diabetes. Moreover it is used in the treatment of patients with type-2 diabetes and its decrease plasma triglyceride [11].

Alpha glucosides inhibitors have evidence helpful for the people with diabetes who haven't able to keep their blood sugar level within a safe



range. In such case inhibitors like acarbose and miglitol help to keep the blood sugar level in safe range by slowing a rate of intestinal which absorbed sugar from blood while eating. These inhibitors can cause low blood sugar while used in combination with other medication for diabetes or with insulin. Diabetic people who are regularly using insulin but once they are use alpha glucosidase inhibitors than they reduce use of insulin [12].

## CHAPTER- 2

### HIGH PERFORMANCE LIQUID CHROMATOGRAPHY.

#### 2. 1 INTRODUCTION: -

Chromatography is the separation of a mixture into single components by using mobile and stationary phase. High performance liquid chromatography is widely use in analytical chemistry and industrial level to identify components. It is one of the fastest growing techniques in pharmaceutical industry for analysis of mixture os substance. It is also knows as high pressure liquid chromatography. It is a high improved as compare to column chromatography. Smaller particle size cans (3-20 $\mu$ ) be analysed by hplc and allows much better separation of a components. Detection method is used in hplc which are highly automated and extremely sensitive as compare to column chromatography [13-14].

Some of the advantage over column chromatography is, (a) small stationary phase are used with widely range available, (b) column which are used in hplc which are made up of metal and small in size so no fear to breakage, (c) hplc is available in analytical and preparative scale. But some of

disadvantage over column chromatography like cost of equipment is high so handle with care, operating pressure is high (500-3000 psi)[14].

Basic principle is based on adsorption. When a mixture of components is introduced into a column, they travel according to their affinity. The components which have a more affinity towards adsorbent, travel slower and vice versa [14].

®NORMAL PHASE HPLC: -

Here, stationary phase is polar in nature and mobile phase is non polar in nature. In this method non polar components eluted first because of less affinity. The column is fitted with a silica gel and hexane used as a solvent. Column length is 150-250 mm and less than 4.6 mm diameter [13-14].

°REVERSE PHASE HPLC: -

Here, stationary phase is non polar and mobile phase is polar in nature. Polar components eluted first. Column size is same but modified to make a non polar by using long chain of hydrocarbons like C8, C12, C4, octadecyl and mixture of water and alcohol used as a solvent. In this case strong attraction between polar solvent and molecular in the mixture passed through column. So it is common phase of hplc [13-14].

2. 2 TYPES OF HPLC TECHNIQUES: -There are major four types of hplc as below,

(1) Partition chromatography: -

In 1952, Archer John Porter Martin and Richard Laurence Millington Syngé were awarded a Noble prize in chemistry to development of these techniques. So it is first techniques which developed for the separation of components like amino acid. These chromatography principles are also applied to the thin layer chromatography, paper chromatography to separates the components.

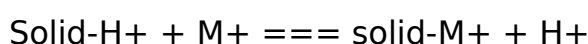
(2) Adsorption chromatography: -

The main hplc principle is based on adsorption. Here components can be separated because of difference in affinity of components towards normal or reverse phases [14].

(3) Ion exchange chromatography: -

It is most frequently used chromatography for the separation and purification of protein, nucleic acid, polypeptides, and other charged molecular. It is a succesful technique because of its high capacity, simplicity, and high resolution power. The main principle is base on reversible exchange of ions between ion present in the solution and those present in ion exchange resin [15].

®Cation exchange:-



(Solution) (Solution)

®Anion exchange: -  $\text{Solid-OH}^- + \text{A}^- \rightleftharpoons \text{solid-A}^- + \text{OH}^-$

(Solution) (Solution). [14]

Separation in ion exchange chromatography depends upon reversible adsorption of charged solute molecular to ion exchange group of opposite charge. Some of the functional group used in ion exchange chromatography is [16],

NAME OF ANION

FUNCTION GROUP

Diethyl amino ethyl

$-O-CH_2-CH_2-N^+H(CH_2CH_3)_2$

Quaternary amino ethyl

$-O-CH_2-CH_2-N^+H(C_2H_5)_2-CH_2CH_2-CH_3$

NAME OF CATION

FUNCTION GROUP

Carboxymethyl

$-O-CH_2-COO-$

SULPHOPROXY

$-O-CH_2-CHOH-CH_2-O-CH_2-CH_2SO_3-$

(4)Size exclusion chromatography: -

It is also known as a gel chromatography. In 1959, Porath and Flodin described the separation of water soluble components by this chromatography. As soon as gel had commercially available, they were extensively used for the separating purpose [17]. Here gel used as molecular sieve and hence mixture of substance with different molecular size are separated. Soft gel like dextran, polyacrylamide are used [14].

It is used to analysis of synthetic polymers and oligomes, lipids, natural macromolecular like protein, glucose, cellulose derivatives, and crude oil alkenes [17].

HPLC techniques also has two different class like (a) analytical HPLC-it is used when analysis very small amount of sample are needed. And (b) preparative HPLC- it is used when separation of few gram of mixture by HPLC. But it is also can classified on base of separation and there are (a) analytical HPLC: - Where only analysis of the samples is done. Recovery of the samples for reusing is normally not done, since the sample used is low. Eg. mg quantities. And other is (b) preparative HPLC: - Where the individual fractions of pure compounds can be collected using fraction collector. The collected samples are reused eg. Separation of few grams of mixtures by HPLC [14].

## 2. 3 INSTRUMENTATION [13-14]: -

Brief introduction of instrumental techniques are as follows,

(1)PUMP: -

Mechanical and pneumatic pump are used in hplc. Mechanical pump operates with constant flow rate where pneumatic pump operates with constant pressure. It's necessary to use pump because solvent must passed through column at high pressure.

## (2) SOLVENT DEGASSING: -

In isocratic techniques, mobile phase are prepared by using mixture of solvents, than passed through column with under high pressure resulting bubble formed, so it cannot give proper result or steady baseline. So before using solvent mixture in HPLC, degassing is necessary. So some of degassing technique is use like ultrasonication.

## HPLC

### (3) COLUMN: -Two types of column used in HPLC,

#### ® Guard column: -

It is also known as safety column. Any impurities present in solvent or mobile phase which can predict by guard column.

#### ®Analytical column: -

It widely use which can decided efficiency of separation. Different column size is available depending upon separation. Column made up of stainless stell, glass, poly ether ether kiton etc.

### (4)DETECTORS: -They are

#### (a)UV detectors: -

<https://assignbuster.com/overview-of-aloe-vera/>

Many organic compounds absorbed UV light of different wavelength. Two type of UV detectors are available depends on wavelength- short wavelength and long wavelength. By UV detector we can get direct reading is how much light is observed.

(b) Fluorometric detectors: - It can be used for the substance which can absorbed fluorescence light.

(c) Conductivity detectors

(d) Refractive detectors

(e) PDA detectors which are similar to UV detectors.

(5) RECORDER: -

They are used to recorded response from detectors. It can record base line and retention time of peak. Now a day computer is used to recording data.

2. 4 HPLC PARAMETERS: -

2. 4. 1 INTERNAL DIAMETER: - Internal diameter of the hplc column play an important role in detection or separation of compounds. For the laboratory use small column was used but in industrial level big column was used.

2. 4. 2 RETENTION TIME: - it is a defined as a time between point of injection and appearance of peak. It measure in minute or seconds.

2. 4. 3 RETENTION VOLUME: - Retention volume in the volume of mobile phase required eluting 50% of the compounds from the column,

Retention time = Retention time. Flow rate.

2. 4. 4 PUMP PRESSURE: - Pumps which are used in hplc vary in pressure depends on separation. Modern HPLC system has been improved to work at high pressure so they are able to separate small particle size from mixture.

2. 4. 5 THEORETICAL PLATE: - It is a functional unit of the column.

Distribution of solute between stationary phase and mobile phase has attained equilibrium in the plate. High of the plate depends on efficiency of separation. It is known as a HETP (HEIGHT EQUIVALENT TO THEORETICAL PLATE). If HETP is less, then column is more efficient and if HETP is more, then column is less efficient.

HETP is given by Van Deemter equation

$$\text{HETP} = A + (B/u) + Cu$$

Where,

A = Eddy diffusion term or multiple path diffusion which arises due to packing of the column. This is unaffected by mobile phase velocity or flow rate. This can be minimised by uniformity in packing.

B = Longitudinal diffusion term or molecular diffusion which depends on flow rate.

C = Effect of mass transfer which depends on flow rate.

u = Flow rate or velocity of the mobile phase.

2. 5 APPLICATION OF HPLC: -

<https://assignbuster.com/overview-of-aloe-vera/>



High performance liquid chromatography has a large divergence of application. Initially it was just used to analysed antibiotic, vitamins by development of reverse phase [18].

High performance liquid chromatography can analysed very minor amount of substance like monograms at analytical scale to micrograms at preparative scale. It is used in a food analysis, biotechnology, biochemical separation, pharmaceutical field, and chemical industries like dyes, fatty acid, toiletry products [14]. High performance liquid chromatography has a easy and good reproducibility so it has been widely use in clinical laboratory. One of the initially application of hplc in the field of clinical was quantification of theophylline in asthmatic infant [18].

Some of important applications are as likes, (a) Isolation of natural product, (b) Quantitative analysis or assay of product, (c) Identify, analysing and purification of compound mixture, (d) To check purity of compound mixture, (e) To find physical properties of compounds, (f) Used in analysing water pollution [18].

The quantity of amount is also determined by hplc which including following method [14],

® By comparing standard and sample peak area, the quantity of sample can be determined which is known as a DIRECT COMPRISION METHOD.

® To find concentration of sample by plotting graph between peak area vs. concentration of the standard drug and intrapolation of unknown sample which is known as CALIBRATION METHOD.

Hplc play an important role in drug discovery with the combination of NMR. It is also useful to identify various chemical species with the combination with mass spectroscopy. It play valuable role in therapeutic drug monitoring to separation of drug from plasma which is quick process [14].

It has some advantages over other chromatography like, (a) Thin layer chromatography is inexpensive but it can't give better resolution to compare HPLC. (b) Mass spectroscopy required a large capacity of maintain budget and skillfull person to run chromatography but in HPLC it's not high budgt. (c) For the HPLC we have large number of mobile phase is available so we have versatility in choice of mobile phase as compare to other chromatography. (d) Atomatic injection system are available in HPLC, so its time saving and give high resolution as compare to other [20].

## 2. 6 LIMITATION OF HPLC: -

It is a time consuming method. Analysis of the compound mixture, mobile phase (mixture of components) are used resulting large amount of waste solution are obtained [14].

## CHAPTER- 3

### EXTERIMENT

#### 3. 1 AIM: -

The aim of project was to known better separation of standard solution of Aloe Vera stem in mobile phase by using isocratic hplc techniques and find enzyme activity on it by using assay whether it is more potent or less potent.

### 3. 2 CHROMATOGRAPHY EQUIPMENTS: -

#### 3. 2. 1 HPLC SYSTEM: -

This system is manufactured by Agilent technology 1200 series with model no G1310A and serial no DE62956545.

#### 3. 2. 2 SOFTWARE USED: -

The software used was Microsoft Windows XP.

#### 3. 2. 3 COLUMN USED: -

The column used was Kromaril 5C18 which is made up of stainless still.

#### 3. 2. 4 APPARATUS USED: -

Pipette with different size available like 1000land 5000 $\mu$ l, Analytical balance, Volumetric flask (10ml, 20ml), Funnel, 100ml Beaker, Measuring cylinder (500ml, 50ml, 20ml, 10ml), Filter paper which made by Fischer scientific with size 0. 45 $\mu$ m, Filter holder made up of pyrex brand glass.

#### 3. 2. 5 PREPARATION OF MOBILE PHASE: -

The mobile phase used was a mixture of a methanol and water with ratio of 50: 50. So take methanol and water, mix well, and filter to remove any solid particle followed by degassing to remove air bubble. Than it is ready for experiment. We are using isocratic system so mobile phase may vary in concentration like, 20: 80, 30: 70.

#### 3. 2. 6 PREPARATION OF STANDARD SOLUTION OF ALOE VERA: -

The standard solution of aloe vera stem was provided by Dr. Solomon Sir and after that I have to dilute in methanol to make up volume to 0.40mg/2ml.

### 3.3 PROCEDURE: -

First set up the HPLC system and run with mobile phase for half an hour. Then inject standard solution of aloe vera stem and run for half an hour, take the graph. Now change the concentration of mobile phase and again repeat same procedure until you get better separation. Then compare all graphs with each other and reach to conclusion. Here we were using two different wavelengths like 280nm and 360nm.

### 3.5 ASSAY: -

Assay technique was used to determine enzyme activity of a substance. Here we were using alpha-glucosidase enzyme. Basic principle involved in assay was, phosphate buffer contains free radical which has no colour but when it comes across with standard solution, retains its colour after incubation. So colour change like colourless to yellow colour took place.

### SOLUTION USED IN ASSAY: -

Here we have to prepare solution for the assay was 25µl, 1.2 U/ml alpha-glucosidase, 25 µl of phosphate buffer, standard solution of aloe vera, 25 µl of 2.5 mM pNPG and finally if proper colour change was not occur then add terminating solution 100 µl of 0.2 M Na<sub>2</sub>CO<sub>3</sub>.

### 3.5.1 PROCEDURE: -

1

2

3

4

E

1

2

3

4

5

6

BLANK

Concentration in raw at no 1-0. 50mg/ml, raw no 2- 0. 25mg/ml, raw no 3- o. 125mg/ml, and raw no 4- 0. 0625mg/ml.

Here all the column from 1 to 6 filled by 25 $\mu$ l, 1. 2 U/ml alpha-glucosidase, then add 25 $\mu$ l of phosphate buffer , add 25 $\mu$ l of test solution, and finally add 25 $\mu$ l of 2. 5 mM pNPG. And then transfer 2. 5 $\mu$ l solution from low concentration to high concentration. Than kept mixture for some time and incubate plate at 37°C for 10 min. and tested absorbance.